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ORIGINAL ARTICLE

Determination of phylogenetic background, fimbrial genes, and antibiotic susceptibility of *Escherichia coli* isolates from urinary tract infections in Bam region, Iran

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Abstract Phylogenetic analysis have shown that *Escherichia coli* (*E. coli*) strains segregate in four main phylogenetic groups A, B1, B2, and D. *E. coli* fimbriae increase invasive capability of bacteria to renal tissues. The purpose of this study was to determine the distribution of phylogenetic groups/subgroups among fimbrial genes and antibiotic resistance patterns of *E. coli* isolates from urinary tract infections (UTI), in Bam (southeast of Iran). A total of 122 *E. coli* isolates from patients with UTI, which were confirmed by biochemical tests, were collected. Antibiotic susceptibility of isolates was examined against six antibiotic agents by disk diffusion method. DNA was extracted and examined for detection of phylogenetic group/subgroups and also for determination of *afaI BC*, *sfa*/*focDE*, and *papEF* genes using PCR technique. *E. coli* isolates were distributed in phylogroups A (45.08 %), D (43.45 %), B2 (7.83 %), and B1 (4.09 %). The examined isolates belonged to six phylogenetic subgroups A_0 (28.69 %), D_2 (24.59 %), D_1 (18.85 %), A_1 (16.39 %), B_{2-3} (7.39 %), and B_1 (4.09 %). Fimbrial genes were found in 27.85 % of isolates. Phylogroups A and D were more prevalent in antibiotic resistance patterns than other phylogenetic groups. The findings of the current study showed that A and D phylogenetic groups were dominant among our isolates. These results differ with that of other researches in other parts of the world. Further studies are required to clarify the phylogenetic background in Bam area. Antibiotic resistant seems to be a common feature of most *E. coli* isolates in this area.

Keywords *Escherichia coli* · Urinary tract infections · Phylogenetic · Fimbriae

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Introduction

The pathogenic strains of *Escherichia coli* (*E. coli*) are predominant causes of abroad diversity of infectious diseases, including urinary tract infections (UTI), septicemia, newborn meningitis, and infections in the central nervous, circulatory, and respiratory systems (Arisoy et al. 2008; Banu et al. 2011). Uropathogenic *E. coli* (UPEC) are serious causative agent of urinary tract infection in both community-based and hospitalized patients. These strains introduce as a subgroup of extraintestinal pathogenic *E. coli* (ExPEC) (Tramuta et al. 2011; Saraylu et al. 2012). It is believed that uropathogenic *E. coli* strains acquire virulence factors including adhesins, hemolysin, aerobactin, cytotoxic necrotizing factor, cytolethal distending toxin, capsule, and uropathogenic-specific protein

as compared with nonpathogenic strains. These virulence factors (VFs) play important roles in the pathogenicity of *E. coli* strains mainly by avoidance of the host defenses system and colonizing the epithelial cells resulting in the invasion to host tissues (Johnson et al. 2001; Ghanbarpour and Akhtardanesh 2012; Farshad et al. 2010).

UPEC strains are able to produce diverse types of adhesins including type 1 fimbriae, P fimbriae (pyelonephritis-associated pili), S fimbriae (*sfa*), and Afa adhesins (*afa*) for afimbrial adhesins that are necessary for the initiation, recognition, and adherence to receptors of the urinary tract cells (Oliveira et al. 2011). Type 1 adhesins (mannose-sensitive hemagglutination) produced by many of pathogenic and nonpathogenic *E. coli* strains. There are large numbers of adhesins such as *pap*, *sfa*, and *afa* that mediate mannose-resistant hemagglutination. These are effective in pathogenicity of *E. coli* strains associated with extraintestinal and intestinal infections (Bouguenec et al. 2001).

Previous studies have shown that *E. coli* strains belong to four major phylogenetic groups (A, B1, B2, and D) and seven subgroups (A_0, A_1, B1, $B2_2$, $B2_3$, D_1, and D_2) (Ghanbarpour et al. 2010; Carlos et al. 2010). ExPEC mostly belonged to B2 group and a trivial extent to D group. Strains of the B2 and D groups carry much virulence factors than strains of the A and B1 groups (Choi et al. 2012; Clermont et al. 2000; Mokracka et al. 2011; Abdallah et al. 2011).

In recent years, indiscriminate use of antibiotics leads to drug resistance in *E. coli* (Rather et al. 2012). There seems to be an increase in the resistance ExPEC to first-line antimicrobial agents such as fluoroquinolones in both hospital environment and community. *E. coli* strains with fluoroquinolone-resistant also show resistance to other antibiotics such as trimethoprim/sulfamethoxazole, gentamicin, tetracycline, ampicillin, and chloramphenicol (Arancibia et al. 2009; Johnson et al. 2005). Trimethoprim–sulfamethoxazole (cotrimoxazole) is common antimicrobial agents recommended for UTI prophylaxis (Cheng et al. 2008). The maximum rate of resistance to trimethoprim–sulfamethoxazole is reported among strains of *E. coli* that cause UTI (Manges et al. 2001).

There are few reports on phylogenetic background of *E. coli* strains from clinical sources in Iran. The aim of this study was to determine the prevalence of phylogenetic groups/subgroups, fimbrial genes, and antibiotic susceptibility of *E. coli* isolates from urinary tract infections in southeast of Iran.

Materials and methods

Bacteria

This study was carried out on 122 *E. coli* isolates from UTI. The isolates originated from urine samples of patients referring to the laboratories of Bam city, Iran. The samples were obtained randomly during July to August 2011. One hundred fourteen of these were from female, and eight were recovered from male patients. The isolates were identified as *E. coli* based on standard bacteriological and biochemical tests. All of the bacterial isolates were stored in Luria–Bertani broth (Invitrogen, Paisley, Scotland), with 30 % sterile glycerol at −80 °C. Two reference strains were used as positive controls for fimbriae genes include A30 (*afaIBC*) and J96 (*sfa*/*focDE*, *papEF*). Reference strains from the ECOR collection were used as controls for phylogenetic grouping: ECOR58 (B1 group), ECOR50 (D group), and ECOR62 (B2 group). Nonpathogenic *E. coli* strain MG1655 was used as a negative control. The reference strains were supplied from Microbiology Department of Ecole Nationale Vétérinaire Toulouse, France.

PCR assay for fimbriae genes and phylotyping

DNA was extracted from *E. coli* isolates and reference strains by lysis method with NaOH. The specific primers used for amplification of the fimbrial genes and phylotyping are presented in Table 1.

E. coli isolates were tested by PCR assay for the presence of *papEF*, *afaIBC*, and *sfa*/*focDE* genes described by Yamamoto et al. (1995). The phylogenetic groups (A, B1, B2, and D) and subgroups (A_0, A_1, B_1, B_{2-2}, B_{2-3}, D_1, and D_2) of each isolate were determined by triplex PCR amplification as described by Clermont et al. (2000).

Antibiotic susceptibility

The susceptibility of all the isolates to six antimicrobial agents was assign by method of disk diffusion according to Clinical Laboratory Standards Institute (CLSI 2012). Commercial antimicrobial disks were supplied from Mast co. UK. The antibiotic disks used in this study were nalidixic acid (30 μg), gentamicin (10 μg), nitrofurantoin (300 μg), ciprofloxacin (5 μg), cotrimoxazole (1.25/23.75 μg), and cephazolin (30 μg).

Statistical analysis

Statistical analysis was carried out using SPSS software version 16. Fisher's exact test was used to evaluate significant differences between fimbriae genes and phylotyping. Positive associations were considered as significant when $p<0.05$.

Results

PCR assays indicated that *E. coli* isolates fell into four phylogenetic groups (A, D, B2, and B1), with prevalence

Table 1 Oligonucleotide primers used in this study

Gene	Primer sequence (5′–3′)	Product size (bp)	Reference
afaIBC	GCT GGG CAG CAA ACT GAT AAC TCT C CAT CAA GCT GTT TGT TCG TCC GCC G	750 bp	Yamamoto et al. (1995)
sfa/focDE	CTC CGG AGA ACT GGG TGC ATC TTA C CGG AGG AGT AAT TAC AAA CCT GGC A	410 bp	Yamamoto et al. (1995)
papEF	GCA ACA GCA ACG CTG GTT GCA TCA T AGA GAG AGC CAC TCT TAT ACG GAC A	336 bp	Yamamoto et al. (1995)
yjaA	TGA AGT GTC AGG AGA CGC TG ATG GAG AAT GCG TTC CTC AAC	211 bp	Clermont et al. (2000)
TspE4C2	GAG TAA TGT CGG GGC ATT CA CGC GCC AAC AAA GTA TTA CG	152 bp	Clermont et al. (2000)
chuA	GAC GAA CCA ACG GTC AGG AT TGC CGC CAG TAC CAA AGA CA	279 bp	Clermont et al. (2000)

of 45.08 (55 isolates), 43.45 (53 isolates), 7.38 (9 isolates), and 4.09 (5 isolates), respectively. Further analysis of PCR phylotyping showed that such isolates fell into five phylogenetic subgroups including A_0 (28.69 %), A_1 (16.39 %), B_{2-3} (7.39 %), D_1 (18.85 %), and D_2 (24.59 %).

Multiplex PCR tests showed that 34 isolates (27.86 %) had at least one of the fimbriae genes. Among the 34 positive *E. coli* isolates, 13 (10.65 %) were positive for *papEF*, 13 (10.65 %) for *afaIBC*, and 8 (6.55 %) for *sfa/focDE* gene.

The positive isolates for virulence genes were distributed in six phylogenetic subgroups, which were presented in Table 2. Presence of *sfa/focDE* in phylogroups were significant (p=0.041). None of the isolates belonged to B_{2-2} phylogenetic subgroup.

According to antibiotic susceptibility test, 117 (95.91 %) isolates were resistance against at least one antibiotic. Antibiogram of isolates showed that maximum rate of resistance (95.91 %) were against cephazolin, whereas most of these isolates belonged to phylogenetic groups A (44.26 %) and D (42.62 %). The minimum range of resistance (5.73 %) was observed against nitrofurantoin. Different ranges of antibiotic resistance were recorded against ciprofloxacin (26.22 %), gentamicin (33.60 %), nalidixic acid (48.36 %), and cotrimoxazole (68.03 %). Phylotyping of 117 antibiotic resistant isolates showed that these isolates belonged to D (D_2 phylogenetic subgroup) and A (A_0 phylogenetic subgroup) groups.

Results showed that 16 antibiotic resistance patterns were observed among the *E. coli* isolates, whereas 5 isolates were sensitive or intermediate for all antibiotics. The highest and lowest antibiotic resistance patterns in relation to phylogenetic background belonged to A (43.44 %) and B1 (3.27 %) phylogenetic groups, respectively (Table 3).

Discussion

This study was done to investigate the prevalence of fimbriae genes (*papEF*, *sfa/focDE*, and *afaIBC*) and antibiotic susceptibility in comparison with phylogenetic groups/subgroups in uropathogenic *E. coli* strains in this part of the world.

The findings of the current study confirmed the complexity of the association between phylogenetic background with fimbriae genes and antibacterial resistance in *E. coli*.

Results showed that the highest prevalence of phylogenetic background were associated with A and D phylogenetic groups, whereas previous studies in different parts of the world found that group B2 and D were the most frequent *E. coli* biotype in UTIs (Mokracka et al. 2011; Abdallah et al. 2011).

Piatti et al. (2008) showed that different geological areas affect the distribution phylogenetic background, virulence genes, and antibiotic resistance of *E. coli* isolates. Therefore,

Table 2 Distribution of fimbrial genes related to different phylogenetic subgroups

Genes	Phylo-group						Total
	A_0 no. (%)	A_1 no. (%)	B_1 no. (%)	B_{2-3} no. (%)	D_1 no. (%)	D_2 no. (%)	
papEF	–	5 (4.09)	–	3 (2.45)	2 (1.63)	3 (2.45)	13 (10.62 %)
sfa/focDE	–	–	1 (0.81)	2 (1.63)	–	5 (4.09)	8 (6.53 %)
afaIBC	3 (2.45)	–	–	–	3 (2.45)	7 (5.73)	13 (10.62 %)

Table 3 Antibiotic resistance patterns in relation to phylogenetic background

Antibiotic resistance patterns	Phylogenetic groups				Total (%)
	A	B1	B2	D	
CIP, CZ, GM, NA, TS	4	–	4	9	17 (13.93)
CIP, CZ, GM, NA	–	–	–	2	2 (1.63)
CIP, CZ, NA, TS	4	–	–	–	4 (3.27)
CZ, NA, NI, TS	–	2	–	–	2 (1.63)
CZ, GM, NA, TS	4	–	–	2	6 (4.91)
CIP, CZ, TS	2	–	–	–	2 (1.63)
CZ, NA, TS	2	–	–	14	16 (13.11)
CZ, NI, TS	2	–	–	–	2 (1.63)
CIP, CZ, TS	2	–	–	–	2 (1.63)
CZ, GM, TS	9	2	–	2	13 (10.65)
CIP, CZ, NA	2	–	–	–	2 (1.63)
CZ, NI	2	–	–	–	2 (1.63)
CZ, GM	–	–	2	2	4 (3.27)
CZ, NA	–	–	–	2	2 (1.63)
CZ, TS	9	–	8	2	19 (15.57)
CZ	11	–	5	6	22 (18.03)
Total (%)	53 (43.44)	4 (3.27)	19 (15.57)	41 (33.60)	117 (95.90)

CIP ciprofloxacin, *CZ* cephazolin, *GM* gentamicin, *NA* nalidixic acid, *NI* nitrofurantoin, and *TS* cotrimoxazole

their survey of isolates in Italy showed that most strains fell into B2, A, and D phylogenetic groups, respectively.

To assess the relationship between fimbrial genes in ExPEC and phylogenetic background, our results showed that 34 isolates possess the virulence genes such as *papEF*, *sfa*/*focDE*, and *afaIBC*. Highest prevalence was in phylogenetic group D (subgroups D_2) and then group A (subgroup A_1), which differs from reports of other studies, which showed VFs such as fimbriae mostly belong to phylogenetic group B2 and to a lesser extent to group D (Clermont et al. 2000; Mokracka et al. 2011; Abdallah et al. 2011). Zhang et al. (2003) have also shown that the presence of pili P among adolescent women was associated with phylogenetic groups B2 and D.

In the present study, pyelonephritis-associated pili (*papEF*) and afimbrial adhesin I (*afaIBC*) (10.65 % both) showed highest prevalence. The prevalence of S fimbriae (*sfa*/*focDE*) among the studied strains was 6.55 %. Two combination patterns which included *papEF*+*sfa*/*focDE* and *sfa*/*focDE*+*afaIBC* (2.45 % both) were detected among the isolates.

Arisoy et al. (2008) showed that a small percentage of *E. coli* isolates were positive for the *pap* (4 %), *afaI* (4 %), *sfa* (1 %), and *sfa*+*pap* (2 %) genes. In another study, Tiba et al. (2008) reported that high prevalence of *E. coli* isolates were positive for *papEF*, *afaIBC*, and *sfa*/*focDE* genes.

In this study, *E. coli* strains containing the *papEF* gene had a higher prevalence in phylogenetic groups D and A, whereas isolates positive for *sfa*/*focDE* and *afaIBC* belonged to group D. The present study showed that phylogenetic distributions of fimbriae genes are inconsistent with previous observations. Most VFs such as fimbrial genes were concentrated predominantly, either within group B2 or jointly within groups B2 and D (Johnson et al. 2001; Abdallah et al. 2011).

Antibacterial resistance in infectious disease is a global public health problem. Resistance among uropathogens to a divers set of antibiotics is increasing. The high levels of resistance of *E. coli* isolates to antimicrobial agents such as cefazolin, cotrimoxazole, nalidixic acid, gentamicin, ciprofloxacin, and nitrofurantoin were observed in this study.

Oliveira et al. (2011) reported that the antibiotic resistances from the highest to the lowest were observed for cotrimoxazole (44 %), nalidixic acid (21 %), gentamicin (16 %), ciprofloxacin (13 %), and cephalothin (4 %).

Different resistance patterns were defined in the 16 categories of isolates. Comparison of resistant isolates with phylogenetic groups showed that most of the examined isolates belonged to D and A phylogenetic groups. Moreno et al. (2006) described that isolates susceptible to the quinolone, fluoroquinolone, and trimethoprim/sulfamethoxazole were significantly associated with phylogenetic group B2, whereas resistant isolates exhibited shifts to non-B2 groups (A, B1, and D). They also had a significantly lower prevalence of virulence genes (*papA*, *hlyA*, and *cnfI*) than did the susceptible isolates.

In conclusion, the present study demonstrates that phylogenetic groups A and D were predominant; VFs such as *papEF*, *afaIBC*, and *sfa*/*focDE* belonged to D phylogenetic group and also *E. coli* isolates resistance to antibiotics shifts to non-B2 phylogenetic groups. The current study showed that P, S fimbriae, and afimbrial adhesin are not the sole pathogenic mechanism on uropathogenic *E. coli*, and there

are many more virulence genes associated with pathogenicity. We need further studies to clarify the role of such virulence genes and the importance of phylogenetic background of *E. coli*.

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